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WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 39/12

A1

(11) International Publication Number: WO 92/15329

(43) International Publication Date: 17 September 1992 (17.09.92)

(21) International Application Number: PCT/US92/01731

(22) International Filing Date: 28 February 1992 (28.02.92)

(30) Priority data:
662,022 28 February 1991 (28.02.91) US

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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: METHODS AND PHARMACEUTICAL COMPOSITIONS FOR INHIBITING PROTEASE FROM HUMAN IMMUNODEFICIENCY VIRUS

(57) Abstract

A method for inhibiting protease from human immunodeficiency virus and for treating viral infections of a host in need thereof which employs a composition containing a copper ion delivery agent. Treatment of the protease of HIV-1 with micromolar concentrations of copper results in complete inhibition of the protease.

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METHODS AND PHARMACEUTICAL COMPOSITIONS FOR INHIBITING PROTEASE FROM HUMAN IMMUNODEFICIENCY VIRUS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to methods for inhibiting human immunodeficiency viruses and pharmaceutical compositions useful therein. More specifically, the present invention relates to methods for inhibiting HIV protease with copper ion, as well as pharmaceutical compositions employing a copper ion delivery agent for use in these methods.

Description of Related Art

The aspartyl protease encoded by the human immunodeficiency virus type 1 (HIV) is essential for the processing of the viral polyproteins encoded by the *gag* and *pol* genes into mature viral proteins. Mutation or deletion of the protease gene blocks replication of the virus, making the protease an attractive target for antiviral therapy of the acquired immunodeficiency syndrome (AIDS). The inhibitors reported thus far are peptides or peptide analogues, some of which were originally studied as inhibitors of other structurally related aspartyl proteases such as pepsin or renin.

Another approach to the inhibition of the protease was suggested by studies of metal-catalyzed oxidation of proteins. Both enzymic and non-enzymic metal-catalyzed oxidation systems are capable of oxidatively inactivating many enzymes. Such systems consist of a redox-cycling metal cation such as copper or iron, a reducing agent, and molecular oxygen. Cytochrome P450/NADPH/O_2 is an example of an enzymic system (Fucci, L. et al., (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1521-1525) while Fe/ascorbate/O_2 is a well-studied non-enzymic system (Levine, R.L. (1983) *J. Biol. Chem.* **258**, 11828-1833). For the latter, it is believed that the iron binds to the enzyme at a specific cation binding site. Oxidation of the reduced form of the metal generates a very reactive oxidizing species, such as the hydroxyl radical. The radical reacts with an amino

acid residue very close to its site of generation, generally inactivating the enzyme. In the case of glutamine synthetase, the site specificity has been studied in detail. Specificity results from the binding of the redox-capable cation to the two binding sites on the enzyme which would normally bind magnesium.

The aspartyl proteases, especially pepsin, have been studied in great detail. The active site of this class of proteases always contains two aspartyl residues, and these could conceivably bind a cation. X-ray crystallographic studies of several aspartyl proteases have employed heavy-metal derivatives (Wlodawer, A. et al., (1989) *Science* **245**, 616-620; Miller, M. et al., (1989) *Nature (London)* **337**, 576-579; and Navia, M. et al., (1989) *Nature (London)* **337**, 615-620) thus establishing the existence of cation binding sites in these proteins. The effect of metal ions on pepsin has been examined by many groups (Lundblad, R. L. et al., (1969) *J. Biol. Chem.* **244**, 154-160; Rajagopalan, T. G. et al., (1966) *J. Biol. Chem.* **241**, 4295-4297; Husain, S. S. et al., (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2765-2768; and Kirchgessner, M. et al., (1976) *Br. J. Nutr.* **36**, 15-22). In general, addition of copper to a pepsin/substrate mixture increases the rate of proteolysis, possibly through an effect on the substrate rather than the enzyme. One report suggested that inhibition of pepsin might be observable under certain conditions (Kirchgessner, M. et al., supra.). However, Lundblad and Stein (Lundblad, R. L. et al., supra.) specifically examined the effect of copper on pepsin and reported that there was no change in the catalytic activity. Copper does accelerate the inactivation of pepsin by diazo compounds but this effect was shown to result from the action of copper on the inhibitor (Lundblad, R. L. et al., supra; Husain, S. S. et al., supra.).

Given this sizeable literature on the aspartyl proteases, direct and specific inhibition of the HIV protease by copper is unexpected.

SUMMARY OF THE INVENTION

Therefore, it is an object of the present invention to provide a method for inhibiting the protease from human immunodeficiency virus.

It is another object of the present invention to provide a method of treating viral infections of a host in need thereof by administrating an antiviral effective amount of a pharmaceutical composition containing a copper ion delivery agent.

It is a further object of the present invention to provide the basis for a pharmaceutical composition for treating viral infections, such as those caused by human immunodeficiency virus.

It is yet another object of the present invention to provide a viral disinfectant composition and viral disinfecting method wherein a disinfectant composition containing a copper ion delivery agent is employed.

The foregoing objects and others are accomplished in accordance with the present invention by providing a method for inhibiting the protease from human immunodeficiency virus which includes administering thereto an antiviral effective amount of a copper ion delivery agent. Another method encompassed by the present invention includes treating viral infections of a host in need thereof wherein an antiviral effective amount of a copper ion delivery agent is administered to the host.

In another embodiment of the present invention, a pharmaceutical composition is provided for treating viral infections of a host in need thereof wherein the composition contains a copper ion delivery agent and a pharmaceutically acceptable excipient.

Further scope of the applicability of the present invention will become apparent from the detailed description and drawings provided below. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and

scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is further described in the accompanying drawings wherein:

Figure 1 illustrates the effect of cations on recombinant wild-type protease activity;

Figure 2 is a time course for inactivation of the recombinant wild-type protease by 25 μm $CuCl_2$;

Figure 3 illustrates the concentration dependence of copper and mercury mediated inactivation of the recombinant wild-type protease; and

Figure 4 illustrates the concentration dependence of copper mediated inactivation of the synthetic mutant protease, its dependence on added thiol, and its lack of dependence on oxygen.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is generally based on the discovery by the inventors that copper potently inhibits the protease from the human immunodeficiency virus-1 (HIV-1). Further, copper inhibition is not oxygen dependent. As evidenced by the results discussed in more detail below, HIV-1 protease is inhibited by micromolar concentrations of Cu^{2+} ions. For example, the protease at 2.5 μM was 50% inhibited by exposure to 5 μM copper ion for 5 minutes while exposure to 25μM caused complete inhibition. Inactivation by Cu^{2+} was rapid and not reversed by subsequent exposure to EDTA nor dithiothreitol. Direct inhibition by Cu^{2+} required the presence of cysteine residue(s) in the protease. Thus, a synthetic mutant protease lacking cysteine residues was not inhibited by exposure to copper ion. However, addition of dithiothreitol as an exogenous thiol rendered even the synthetic mutant protease susceptible to inactivation by copper ion. Oxygen was not required for inactivation of either the recombinant wild-type nor synthetic mutant protease.

In the method and pharmaceutical composition of

the present invention, a copper ion delivery agent is employed for delivery of copper ions to the target protease thereby causing inhibition thereof. Generally, examples of appropriate copper ion delivery agents include copper salts, metal chelators, and peptides and proteins which bind copper. More preferred examples of these agents include copper chloride; EDTA, *o*-phenanthroline, histidine, and desferrioxamine; histidine-containing peptides, ceruloplasmin, and albumin. The copper ion delivery agent should possess molecular characteristics which allow it to specifically deliver the copper ion to the protease and which in itself would be inhibitory; thus the delivery agent and the copper may act synergistically. Examples of this preferred class of targeted delivery agents are peptide inhibitors of the protease, including pepstatin-type inhibitors. The copper ion delivery agent may also be combined with or administered in the presence of other components, such as thiols, for example, dithiothreitol, cysteine, glutathione, and their esters and other derivatives.

In another embodiment of the present invention, the pharmaceutical composition may contain other pharmaceuticals in conjunction with the copper ion delivery agent, wherein the pharmaceuticals are used to therapeutically treat acquired immunodeficiency syndrome (AIDS). Representative examples of these additional pharmaceuticals include antiviral compounds, immunomodulators and immunostimulants, and antibiotics. Exemplary antiviral compounds include AZT, ddI, ddC, gancyclovir, fluorinated dideoxynucleotides, etc. Exemplary immunomodulators and immunostimulants include various interleukins, CD4, cytokines, antibody preparations, blood transfusions, cell transfusions, etc. Exemplary antibiotics include antifungal agents, antibacterial agents, anti-Pneumcysitis carnii agents, etc.

Since the copper ion delivery agent of the present invention is capable of inhibiting and inactivating viruses, it may also be employed as an active ingredient

in a viral disinfectant along with a suitable carrier. The viral disinfection composition should contain an adequate concentration of the copper ion delivery agent necessary for disinfecting a desired target from viruses, such as HIV type viruses.

The method and composition of the present invention may be employed in the treatment of a variety of viruses including, for example, members of the HTLV family, especially human immunodeficiency viruses HIV-1 and HIV-2, as well as other protease-dependent retroviruses such as HTLV-1 and animal leukemia viruses.

Inhibition by Copper and Mercury. Pepsin and other aspartyl proteases are generally not inhibited by divalent cations, including copper (Lundblad, R. L. et al., supra.). However, our studies of the susceptibility of the HIV protease to metal-catalyzed oxidation led to the discovery that micromolar concentrations of copper or mercury caused marked inhibition of the enzyme.

Fig 1. shows the effect of cations on recombinant wild-type protease activity. Enzyme (2.5μM) was incubated with 25μM cation in 150 mM sodium acetate, pH 5.5, containing 10% (v/v) glycerol for 5 minutes at 37°C. The assay for activity was then begun by the addition of substrate in 150 mM sodium actetate/6 mM EDTA, yielding a final concentration of 1 mM EDTA. The incubation was stopped after 20 minutes additional incubation. Al and Cr were trivalent; Ca, Co, Mg, Mn, Ni, Pb, Zn, Hg, and Cu were divalent; K was monovalent.

Addition of the chelator EDTA immediately after adding the metal could not prevent inhibition. When EDTA was added just before the metal, inhibition by copper was blocked but not inhibition by mercury.

Both cations have high affinity for amino acids and might be inhibiting proteolysis by binding either to the protease or to the nine-residue peptide substrate. Since protease is present at micromolar concentration and peptide at millimolar, the protease was the more likely target. However, a metal-peptide complex could still be

the inhibitory species. The protease was shown to be the actual target of copper ion inhibition by incubating either the peptide or the protease with 25 μM copper for 5 min. Then EDTA was added to 1 mM, followed by protease or peptide to provide a complete assayable system. Preincubation of the peptide caused no inhibition of activity while preincubation of the protease led to virtually complete loss of enzymatic activity as shown below in Table 1.

Table 1. Effect of order of addition upon inhibition of the recombinant wild-type HIV protease.

1st Addition	2nd Addition	3rd Addition	Activity, units
Protease + Cu^{2+}	EDTA	Substrate	0.02
Substrate +Cu^{2+}	EDTA	Protease	1.61
Protease	EDTA	Substrate	1.28

Mixtures were incubated 5 min at 37°C between additions. The final concentration of $CuCl_2$ was 25 μM and that of EDTA was 1 mM.

Inhibition is rapid, as shown in Fig. 2 which illustrates the time course for inactivation of the recombinant wild-type protease by 25μM $CuCl_2$. Protease (2.5 μM) was incubated with (●) or without (■) copper in 150 mM sodium acetate/10% (v/v) glycerol, pH 5.5 at 37°C. The activity assay was initiated by transferring 10 μl of the incubation solution to a separate tube containing 2 μl substrate in 150 mM sodium acetate with 6 mM EDTA.

The concentration dependence of inhibition is plotted in Fig. 3 which shows concentration dependence of copper (●) and mercury (■) mediated inactivation of the recombinant wild-type protease. Protease was incubated with the cation for 5 minutes and then assayed as described with regard to Fig. 1.

The affinity of binding has not yet been

determined so one cannot deduce the stoichiometry of binding from the concentration dependence. However, if one assumes that binding is very tight (stoichiometric), then the minimal requirement for inhibition is the binding of about 1 mercury cation per protease subunit or about 2 copper cations. Further, the binding of the first copper does not appear to affect activity.

Involvement of Thiols. Copper and especially mercury tend to bind to the sulfhydryl group of cysteine residues. The HIV protease has two Cys residues. Cys_{67} lies on the surface of the enzyme while Cys_{95} participates in forming the dimer interface of the active protease. Treatment with dithiothreitol restored at least 80% of the activity of the mercury-inhibited enzyme as shown below in Table 2. Although some forms of the viral protease may lack cysteine residues, such as for example HIV-2, inactivation still occurs if the thiol is supplied exogenously. Therefore, a thiol may be co-administered with the copper ion delivery agent or the latter administered in the presence of a thiol to achieve desired protease inhibition. Also, normal plasma and cell content of thiols may be adequate to support copper inhibition properties.

Table 2. Restoration of protease activity

1st Addition	2nd Addition	3rd Addition	4th Addition	Activity, % of Control
None	None	None	Dithiothreitol	100
None	None	Dithiothreitol	None	100
Cu^{2+}	None	Dithiothreitol	None	24
Hg^{2+}	None	Dithiothreitol	None	82
Hg^{2+}	EDTA	Cu^{2+}	Dithiothreitol	74
Hg^{2+}	Cu^{2+}	EDTA	Dithiothreitol	78
Cu^{2+}	Hg^{2+}	EDTA	Dithiothreitol	23
Cu^{2+}	Hg^{2+}	Dithiothreitol	None	19

The protease was the wild-type. One μl additions were made to 19 μl enzyme solution, followed by 2.5 min incubation at 37°C. Stock solutions used for the additions were 500 μM $CuCl_2$, 500 μM $HgCl_2$, 200 mM dithiothreitol, and 20 mM EDTA. One μl water was substituted when the addition was "none". Activity was assayed for 20 minutes following the final incubation. The assay was initiated by adding substrate in 4 μl 150 mM sodium acetate, pH 5.5, containing 6 mM EDTA.

Treatment of the copper-inhibited enzyme with dithiothreitol gave a variable, but low recovery of 10-30%. Nevertheless, the copper-treated enzyme had not been irreversibly inhibited. Refolding of the enzyme from 6 M guanidine restored activity to the same level as that of a control not treated with copper.

The ability of one metal to displace the other from the enzyme was then examined by taking advantage of the observation that the mercury-inhibited enzyme was reactivated by treatment with dithiothreitol while the copper-inhibited enzyme was not. Neither metal appeared able to displace the other during the 2.5 minutes incubation (Table 2). This result suggests that the off-rate of bound metal is slow, consistent with the observed high affinity of binding.

Protease was also titrated with the sulfhydryl reagent 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman's reagent), although the accuracy of the determination was limited by the amount of protein available. As shown in Table 3 below, untreated protease had about 2 titratable sulfhydryls per subunit under denaturing conditions. Copper-inhibited protease showed a small decrease in sulfhydryl groups while mercury-inhibited protease lost most of its titratable sulfhydryls.

Table 3. Titratable sulfhydryl groups in the recombinant wild-type protease.

Protease Treatment	$\Delta A_{412\ nm}$	SH / Subunit
Experiment 1:		
None	0.049	1.8
100 μM Cu^{2+}	0.034	1.2
100 μM Hg^{2+}	0.014	0.5
Experiment 2:		
None	0.032	1.4
100 μM Cu^{2+}	0.019	0.8
100 μM Hg^{2+}	0.000	0.0

The protease concentration was 2.0 μM in the first preparation and 1.64 μM in the second.

The role of cysteine residues in mediating copper inhibition might be probed with protease in which the cysteines were alkylated. However, as reported by Meek and colleagues (Meek, T. D. et al., (1989) Proc. Natl. Acad. Sci. USA 86, 1841-1845), the carboxyamidomethylated enzyme was catalytically inactive, so that the effect of copper could not be evaluated. However, the essential role of cysteine was demonstrated by investigation of a variant protease which lacked cysteine residues. This variant enzyme was produced by solid-phase synthesis, with the two cysteine residues replaced by α-amino butyric acid (Schneider, J. et al., (1988) Cell 54, 363-368). Copper and mercury did not inhibit this variant protease as shown below in Table 4.

Table 4. Effect of oxygen and dithiothreitol on protease activity

Protease	Metal	Dithiothreitol	Oxygen	Activity, % of Control
Wild-type	Cu^{2+}, 25 μM	-	-	0
Wild-type	Cu^{2+}, 25 μM	-	+	0
Synthetic	-	+	+	98
Synthetic	Cu^{2+}, 100 μM	-	-	98
Synthetic	Cu^{2+}, 100 μM	-	+	98
Synthetic	Cu^{2+}, 100 μM	+	-	3
Synthetic	Cu^{2+}, 100 μM	+	+	3
Synthetic	Hg^{2+}, 100 μM	-	+	93

When added, the concentration of dithiothreitol was 10 mM.

Effect of Oxygen. The discrepancy of recovery of activity upon dithiothreitol treatment was curious. It seemed likely that both copper and mercury inhibited the protease by direct binding. Addition of dithiothreitol to the inhibited enzyme should reverse the inhibition, if the reactions were relatively fast. However, it is known that iron or copper/ thiol/ oxygen form a potent metal-catalyzed oxidizing system capable of inactivating many enzymes. Thus, addition of dithiothreitol to the copper-treated enzyme might also cause oxidative modification of the protease.

This possibility was considered in experiments which examined the effects of oxygen and dithiothreitol on the inhibition of the recombinant wild-type protease and

on the variant protease which lacked cysteine residues. Either anaerobic or aerobic incubation of the recombinant wild-type protease with copper caused loss of enzymatic activity (Table 4). Incubation of the synthetic protease with copper alone had no effect on proteolytic activity.

However, the addition of dithiothreitol revealed a copper-dependent inactivation which did not require either oxygen or cysteine (Table 4). That is, the synthetic protease was inactivated when incubated with copper and dithiothreitol, even in the absence of oxygen. No significant inactivation occurred when the synthetic protease was exposed to reduced dithiothreitol alone, nor to oxidized dithiothreitol with or without copper.

The extent of inactivation by copper and dithiothreitol varied with the concentration of copper. Figure 4 illustrates the concentration dependence of copper mediated inactivation of the synthetic mutant protease its dependence on added thiol, and its lack of dependence on oxygen. Enzyme (4.3 μM) was incubated with the cation for 5 minutes, after which either dithiothreitol or water was added. Following an additional 5 minutes incubation, protease activity was assayed as described in Fig. 1. Incubations were aerobic (squares) or anaerobic (circles). Open symbols represent incubations without dithiothreitol and closed symbols with 10 mM dithiothreitol. This observation was consistent with several possible mechanisms of inactivation, the simplest of which would be direct inhibition by Cu^{1+}, produced through reduction of Cu^{2+} by dithiothreitol. However, aerobic exposure of the protease to 100 μM CuCl for 10 minutes had no effect on proteolytic activity. Thus, neither the cuprous nor cupric form of copper alone were capable of inhibiting the synthetic protease. However, addition of 10 mM dithiothreitol during the last 5 minutes incubation with CuCl caused complete loss of activity, paralleling the results with $CuCl_2$.

EXAMPLES

MATERIALS AND METHODS

HIV-1 protease. Production, purification, and assay of the recombinant wild-type protease in *Escherichia coli* were as described (Boutelje, J. et al., (1990) *Arch. Biochem. Biophys.* 283, 141-149). The chemically synthesized protease (Schneider, J. et al., supra.), a generous gift from Dr. Steven Kent, was obtained as a lyophilized powder. Before use, this synthetic protease was dissolved in 6 M guanidine HCl, 50 mM Tris (pH 7.8), 5 mM DTT, and 1mM EDTA and refolded as described below. Both the recombinant and the synthetic protease were stored at -70°C in 20 mM HCl at protein concentrations of 100-200 μg/ml.

Anaerobic experiments were performed in the Anaerobic Laboratory of the National Institutes of Health (Poston, J. M. et al., (1971) *Methods Enzymol.* 22, 49-54). The atmosphere in this laboratory was constantly monitored and never exceeded an oxygen content of 5 ppm. After entry into the anaerobic room, protease and peptide solutions were pump purged ten times and buffers were sparged with purified argon for at least 10 minutes.

Protease was refolded as follows. First, the enzyme was dialyzed against 6 M guanidine HCl, 50 mM Tris (pH 7.8), 1 mM EDTA, 5 mM dithiothreitol at ambient temperature for 2 hr. The enzyme solution was dialyzed next at 4°C against 3 M guanidine HCl, 50 mM Tris (pH 7.8), 1 mM EDTA, 1 mM dithiothreitol for 2 hr, followed by an additional 2 hr dialysis against 1 M guanidine HCl, 50 mM Tris (pH 7.8), 1 mM EDTA, 1 mM dithiothreitol. The final dialysis was into 20 mM HCl, with an additional change of the HCl solution before overnight dialysis. The dialysis tubing (Spectrum Medical Industries, Los Angeles, CA) had a nominal molecular weight cutoff of 6,000-8,000. The ratio of protease volume to dialysate was 1:100 for guanidine solutions and 1:2000 for the HCl.

Cation Inhibition. Stock solutions of 1 M cations were made by dissolving the salts in water with acidification by HCl to pH 3 - 5. Typically, protease (2.5 μM) was

incubated with 25-100 μM cation for 5 minutes at 37° in 10 μl 150 mM sodium acetate, pH 5.5, containing 10% (v/v) glycerol. The assay was started by adding substrate in 2 μl of 150 mM sodium acetate (pH 5.5), 6 mM EDTA, yielding 1 mM EDTA in the assay solution. After 20 minutes at 37°C, products were quantitated by high pressure liquid chromatography as previously described (Boutelje, J. et al., supra.).

Sulfhydryl Titration. Ellman's reagent, 5,5'-dithiobis(2-nitrobenzoic acid) was used to quantitate sulfhydryl groups in protein denatured in 6M guanidine HCl. To one volume of protease in 150 mM sodium acetate, pH 5.5, was mixed three volumes of 1.3 mM 5,5'-dithiobis(2-nitrobenzoic acid) in 8 M guanidine HCl, 133 mM Tris, and 13.3 mM EDTA giving a final pH of 7.4. After mixing, the solution was incubated at 37°C for 5 minutes. The absorbance of the 2-nitro-5-thiobenzoate was measured at 412 nm against a reagent blank in a Hewlett Packard model 8450 spectrophotometer. The concentration of the sulfhydryl groups was calculated using a molar absorption coefficient of 13,700 for the dianion (Riddles, P. W. et al., (1979) *Anal. Biochem.* **94**, 75-81.).

The carboxyamidomethyl-cysteine derivative of the protease was prepared by treatment with iodoacetamide (Means, G. E. et al., (1971) *Chemical Modification of Proteins* (Holden-Day, Inc., San Francisco, CA), pp. 105-138). To assure reduction of the cysteine residues, one volume of enzyme in 20 mM HCl was mixed with three volumes of 8 M guanidine HCl, 133 mM Tris, 13.3 mM EDTA giving a final pH of 8.0, and then incubated with 5 mM dithiothreitol for 15 minutes at 37°C. The solution was then made 20 mM in iodoacetamide, incubated at room temperature for 2 hr in the dark, and quenched with excess dithiothreitol (10 mM). The sample was then dialyzed into 20 mM HCl as described for enzyme refolding.

Analytical Methods. The protease concentration was calculated from the absorbance at 280 nm, corrected for light scatter (Levine, R. L. et al., (1982)

Biochemistry **21**, 2600-2606), using molar absorptivities calculated (Mihalyi, E. (1968) J. Chem. Eng. Data **13**, 179-182) from the sequence of the protease (ϵ=12,300). The accuracy of this method was confirmed by amino acid analysis after acid hydrolysis of the protease. Oxidized dithiothreitol was prepared by stirring a solution of reduced dithiothreitol in room air overnight. Oxidized and reduced dithiothreitol were quantitated by high pressure liquid chromatography with monitoring at 210 nm. These compounds are well-separated from the products and substrate of the protease assay and could therefore be quantified using the same analytical system as for the protease assay (Boutelje, J. et al., supra.).

The copper ion delivery agents employed in the present invention may be made into pharmaceutical compositions by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms such as tablets, capsules, powders, granules ointments, solutions, suppositories, injections, inhalants, and aerosols in the usual ways for their respective route of administration. The following methods and excipients are merely exemplary and are in no way limiting.

In pharmaceutical dosage forms, the copper ion delivery agents employed in the present invention may be used in the form of their pharmaceutically acceptable salts and other compounds, and also may be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds.

In the case of oral preparations, the copper ion delivery agents may be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, e.g. with conventional additives such as lactose, mannitol, corn starch or potato starch; with binders such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants such as

talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

Furthermore, the copper ion delivery agents employed in the present invention may be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases.

The copper ion delivery agents employed in the present invention may be formulated into preparations for injections by dissolving, suspending or emulsifying them in an aqueous or non-aqueous solvent, such as vegetable oil, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

In the cases of inhalations or aerosol preparations, the copper ion delivery agents employed in the invention in the form of a liquid or minute powder may be filled up in an aerosol container with gas or liquid spraying agents, and if desired, together with conventional adjuvants such as humidifying agents. They may also be formulated as pharmaceuticals for non-pressured preparations such as in a nebulizer or an atomizer.

A suitable dosage is that which will result in concentration of the copper ion delivery agents in blood and/or tissues harboring the virus which are believed to inhibit the virus. The preferred dosage is that amount sufficient to render a host free of the particular viral infection. The dose may vary when the compounds are used prophylactically. The dose may be adjusted to provide the concentration to bind two copper ions to the protease in order to assure complete inhibition.

Unit dosage forms for oral administration such as syrups, elixirs, and suspensions wherein each dosage unit, e.g., teaspoonful, tablespoonful, contains a predetermined amount of the copper ion delivery agents employed in the present invention can be by a pharmaceutically acceptable

carrier, such as Sterile Water for Injection, USP, or by normal saline.

The copper ion delivery agents employed in the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

The copper ion delivery agents employed in the present invention may also be administered transdermally when combined with appropriate carriers, such as for example dimethylsulfoxide.

The copper ion delivery agents employed in the present invention can be utilized in aerosol formulation to be administered via inhalation. The copper ion delivery agents employed in the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the copper ion delivery agents calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable, diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

The pharmaceutically acceptable excipients, for example, vehicles, adjuvants, carriers or diluents are readily available to the public.

Any necessary adjustments in dose can be readily made to meet the severity of the infection and adjusted accordingly by the skilled practitioner.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the

spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for treating viral infections of a host needing treatment which comprises administering to the host an antiviral effective amount of a composition comprising a copper ion delivery agent and a pharmaceutically acceptable excipient.

2. The method of claim 1, wherein said viral infection is caused by human immunodeficiency virus.

3. The method of claim 1, wherein said copper ion delivery agent is a copper salt.

4. A method for inhibiting protease from a virus by administering thereto an effective amount of a composition containing a copper ion delivery agent and a pharmaceutically acceptable excipient.

5. The method of claim 4, wherein said copper ion delivery agent is a copper salt.

6. The method of claim 4, wherein the amount of composition administered is sufficient to effect inhibition of human immunodeficiency viruses.

7. A pharmaceutical composition for treating viral infections caused by human immunodeficiency virus which comprises a copper ion delivery agent and a pharmaceutically acceptable excipient.

8. The method of claim 7, wherein said copper ion delivery agent is a copper salt.

9. The method of claim 1, wherein said copper ion delivery agent is selected from the group consisting of copper salts, metal chelators, peptides and proteins which bind copper.

10. The method of claim 4, wherein said copper ion delivery agent is selected from the group consisting of copper salts, metal chelators, peptides and proteins which bind copper.

11. The composition of claim 7, wherein said copper ion delivery agent is selected from the group consisting of copper salts, metal chelators, peptides and proteins which bind copper.

12. The method of claim 1, wherein said

composition further comprises a thiol component.

13. The method of claim 4, wherein said composition further comprises a thiol component.

14. The composition of claim 7, wherein said composition further comprises a thiol component.

15. The composition of claim 7, wherein the copper ion delivery agent is present in an amount sufficient to inhibit human immunodeficiency viruses.

16. A viral disinfectant composition for disinfecting a desired target from viruses which comprises a copper ion delivery agent and a suitable carrier.

17. A method for disinfecting a virally infected target which comprises administering to the target an effective amount of a viral disinfectant composition containing a copper ion delivery agent and a suitable carrier.

18. The composition of claim 7, further comprising a drug selected from the group consisting of antiviral compounds, immunomodulators, immunostimulants and antibiotics.

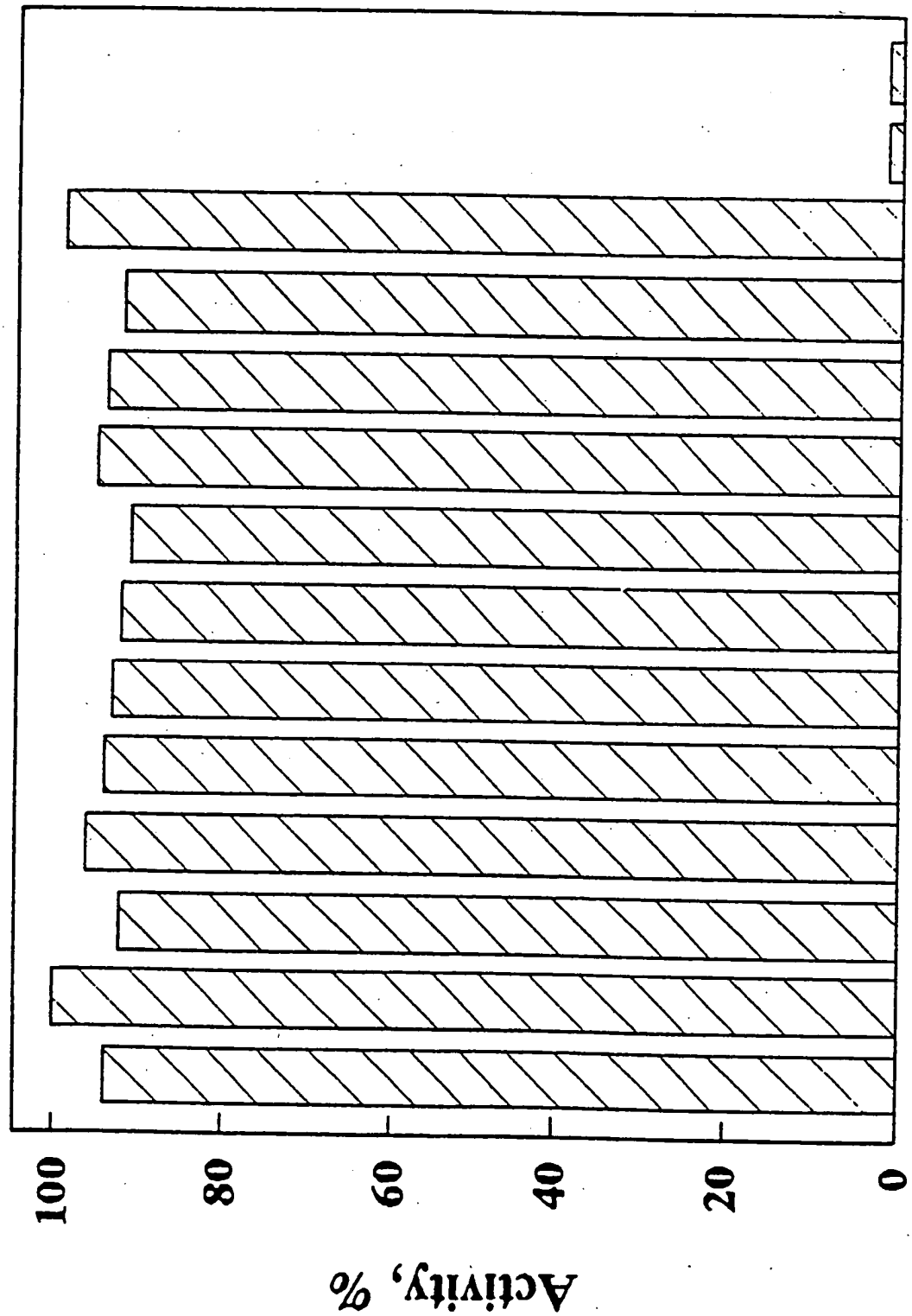

FIG. 1

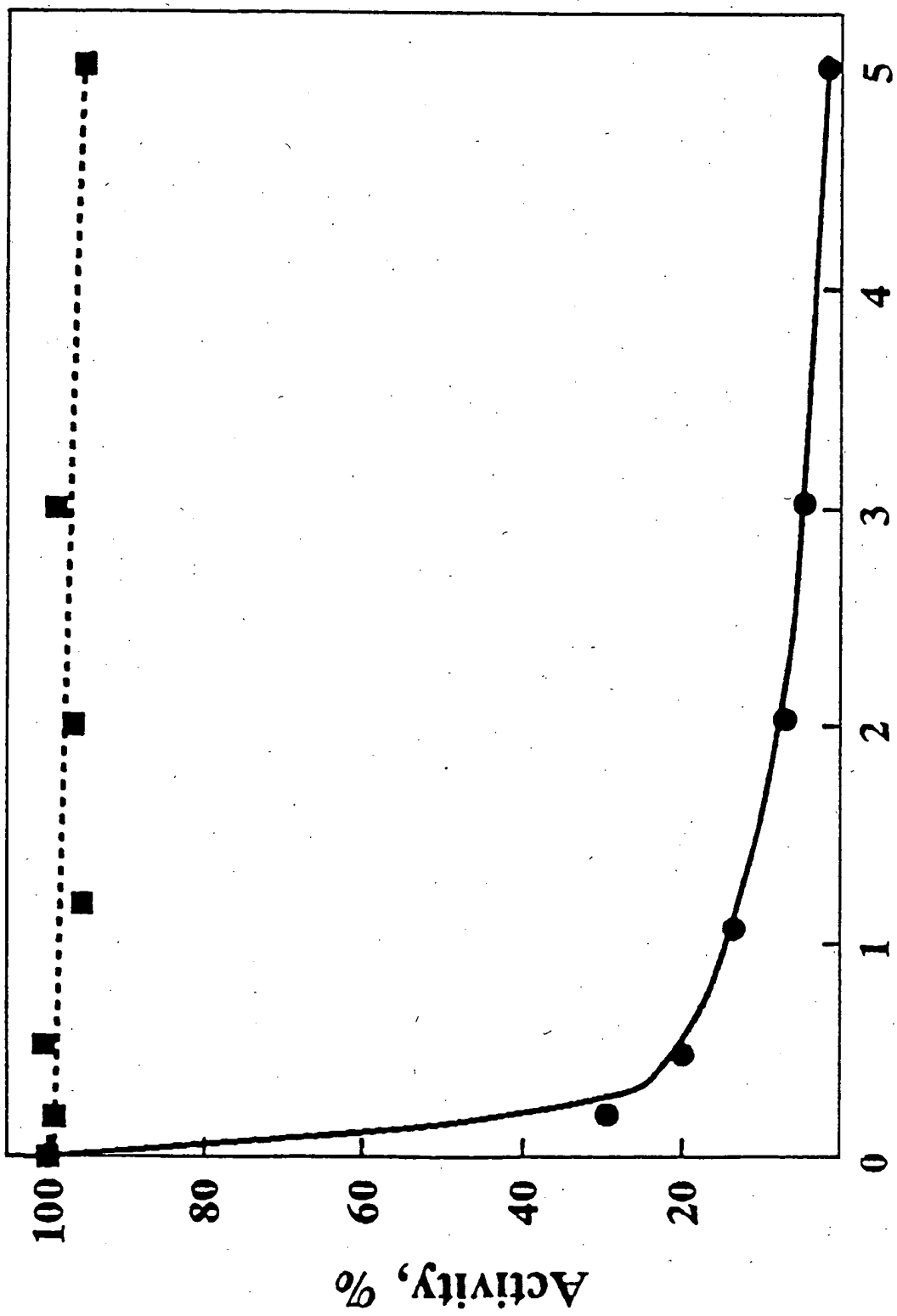

FIG. 2

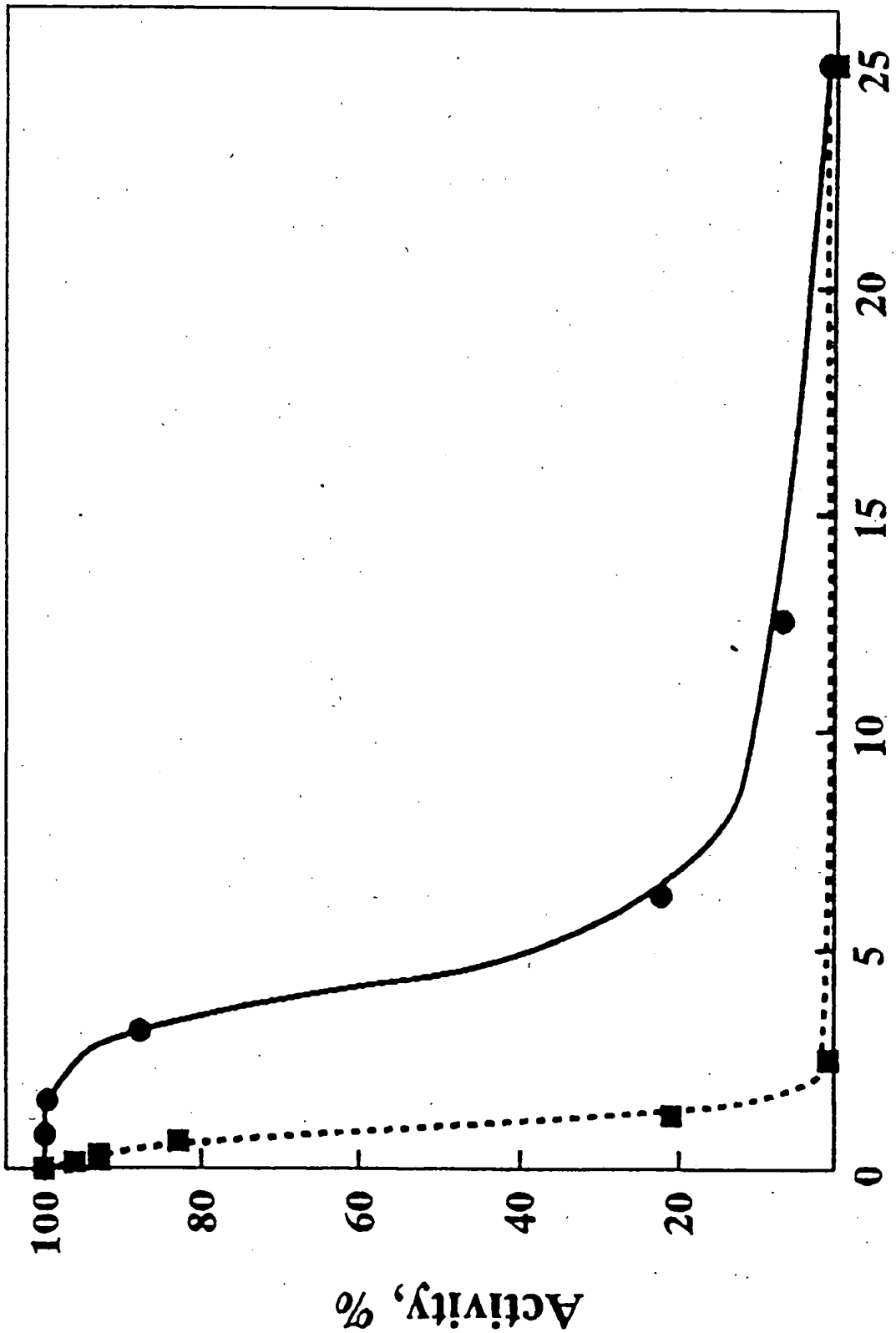

FIG. 3

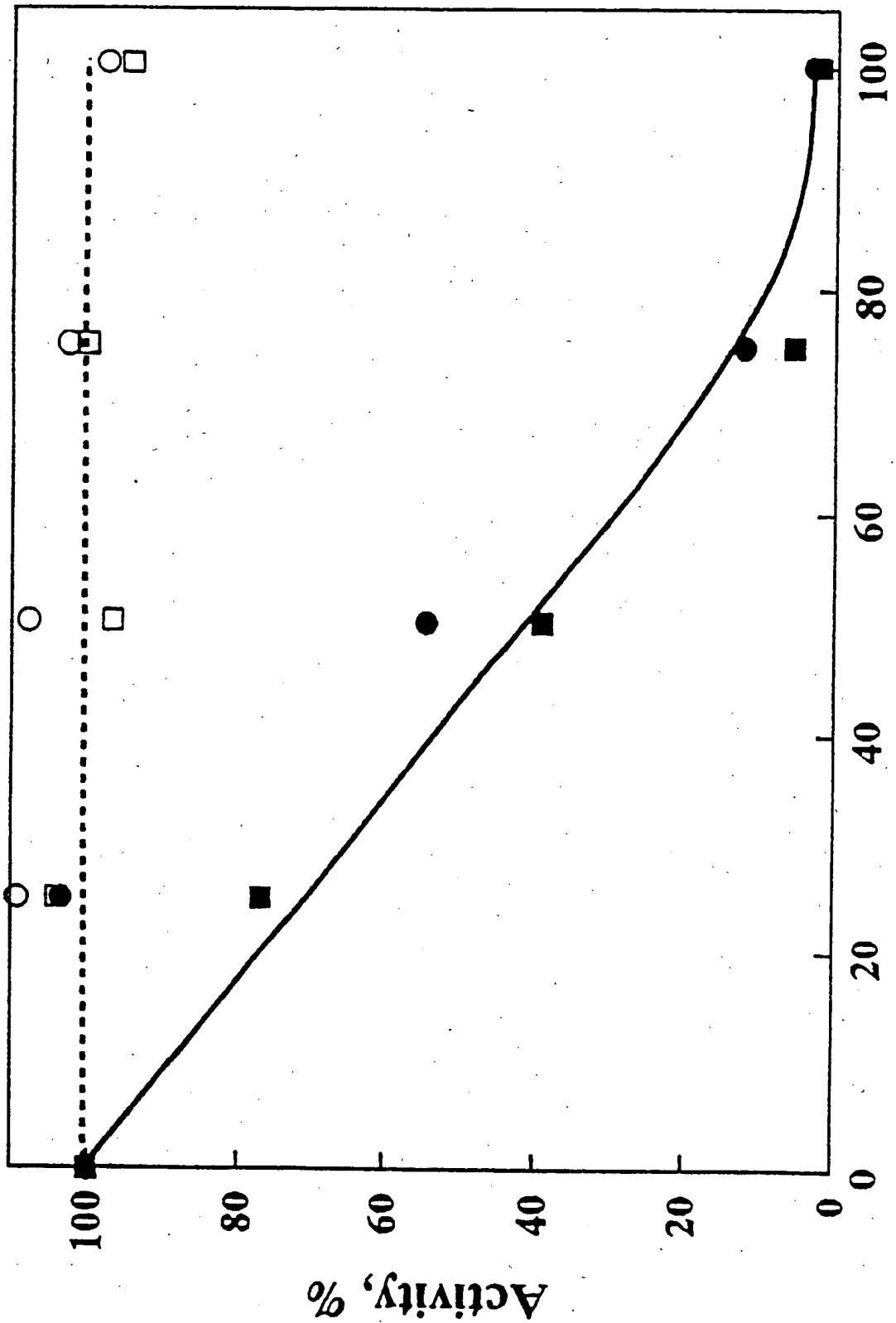

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/01731

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5) : A61K 39/12
US CL : 424/89

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	424/89

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

Dialog (files 155,154, 157,357,5,399), APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y	US,A,7315911 No. 90-091329/12 (Rabinovitz, et al.) 23 January 1990, see abstract	1,2,4,6,7,9-18
Y	Nature, volume 329, issued 15 October 1987, Katoh, et al., "Inhibition Of Retroviral Protease Activity By An Aspartyl Proteinase Inhibitor", pages 654-656, see entire document.	4,6,7,10,11
Y	Journal of Virology, Vol. 63, No. 5, issued May 1989, Katoh, et al., "Retrovirus Protease Characterized As A Dimeric Aspartic Proteinase", pages 2226-2232, see abstract.	1-11,15-18
Y	Cell, volume 54, issued 29 July 1988, Schneider, et al., "Enzymatic Activity Of A Synthetic 99 Residue Protein Corresponding To The Putative HIV-1 Protease", pages 363-368, see abstract.	1-11, 15-18
Y	JP, A, 62252777 No. 87-351025/50 (TAIHO PHARM) KK 04 November 1987, see abstract	1-11, 15-18

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
30 April 1992	15 MAY 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	Richard Fraud for Lynette F. Smith

Form PCT/ISA/210 (second sheet)(May 1986) ß